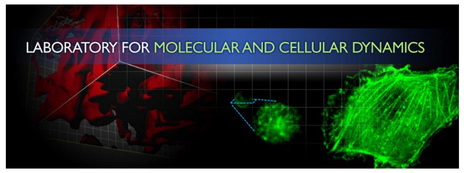
Synergistic interactions between TGF-β1- and TNFα-induced signaling in cancer cells are the result of TAK1- and Smad7-mediated crosstalk



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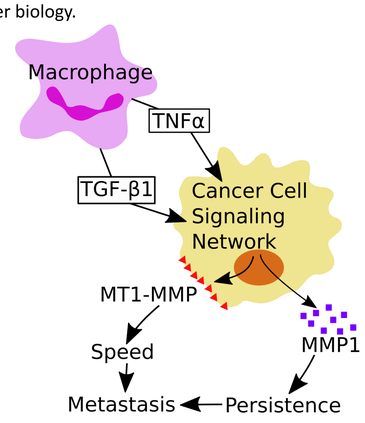
Abstract

A tumor is not a homogenous mass of cancer cells, but is in fact a diverse microecosystem populated by many physical, chemical, and biological actors, all of which interact with each other and, together, drive gross tumor behavior. When small signaling molecules known as cytokines are expressed and secreted from a cell into the extracellular space, they can bind to corresponding receptors on the same cell or other cells, initiating intracellular signaling pathways capable of affecting many cell processes and behaviors. Two cytokines expressed and secreted by tumor-associated macrophages (TAMs), transforming growth factor-β1 (TGF-β1) and tumor necrosis factor-α (TNFα), have been shown to modulate the speed and directedness of cancer cell migration, as mediated by changes in the extracellular matrix (ECM)-degrading enzymes membrane-type-1 matrix metalloproteinase (MT1-MMP) and matrix metalloproteinase-1 (MMP1), respectively. These expression changes—and thus the migration effects—are driven by a nonlinear signaling network characterized by extensive crosstalk between the downstream intracellular signaling pathways activated by these cytokines, where migration directedness is controlled by a synergistic integration of TGF-β1 and TNFα activity and migration speed is more directly regulated by TGF-β1 activity alone. In order to elucidate the intracellular signaling mechanisms and species responsible for these behaviors, we have constructed an ordinary differential equation signaling model describing the TGF-β and TNFα signaling pathways in cancer and how they interact, and used this model to reproduce and explore the mechanisms underlying the observed synergistic interaction between the two pathways. From this computational analysis of these pathways, we determined the connection points between the TGF-β and TNFα signaling pathways that facilitate this behavior, demonstrated the ability of our model to reproduce experimental observations, explored the mechanisms underlying this ability, and showed that in the absence of these mechanisms the observed signaling behavior cannot be recaptured. In particular, we showed how TGF-β-activated kinase 1 (TAK1), an intermediate signaling protein indirectly activated by both TGF-β1 and TNFα, serves as an integrator of TGF-β and TNFα signaling, and Smad7, a transcriptionally-regulated signaling protein, serves as a mutually regulated inhibitor of both pathways, facilitating the observed signaling. Finally, we conducted sensitivity analyses to explore other signaling species exerting significant control over cytokine-regulated MMP expression. By analyzing this system through mathematical modeling methods, we hope to gain a broader understanding of how TAM-induced cytokine signaling affects cancer cell behavior and demonstrate the utility of these methods in cancer biology.

Prior Experimental Motivation

Fig. 1. Experimental data suggests that macrophage-secreted TGFB1 and TNFα modulate cancer cell migration and persistence, with both cytokine signaling networks interacting with each other [1]. These interactions allow the emergence of unique phenomena such as the synergistic heightened expression of MMP1 in the presence of both TGF-β1 and TNFα.



Hypothesized Network

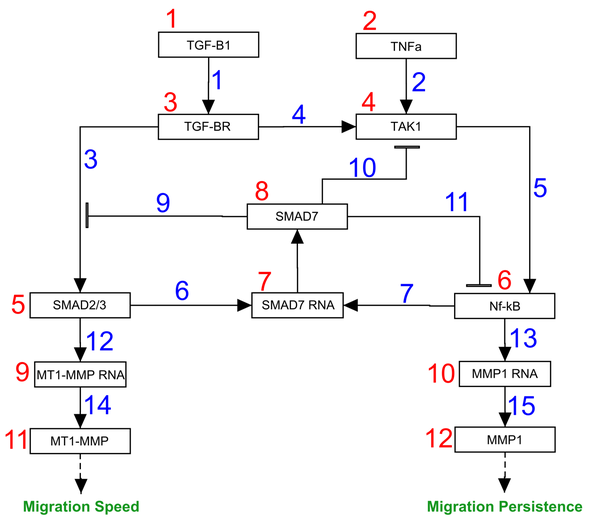


Fig. 2. Proposed network as modeled. All species are numbered in red, and all reactions are numbered in blue. Note that the edges on this network map do not always represent single biological reactions but may represent composites of multiple linearly arranged reactions.

Modeling Framework

System of ODEs describing the concentrations of 12 signaling species over time:

- S1, TGF-β1: $\frac{dS_1}{dt} = 0$
- S2, TNFα: $\frac{dS_2}{dt} = 0$
- S3, TGF-βR complex: $\frac{dS_3}{dt} = p_1 S_1 (S_{3,tot} - S_3) - d_P S_3$
- S4, TAK1: $\frac{dS_4}{dt} = p_2 S_2 S_3 (S_{4,tot} - S_4)(1 - S_8/(k_{10} + S_8)) + S_{4,0} - d_P S_4$
- S5, Smad2/3: $\frac{dS_5}{dt} = p_3 S_3 (S_{5,tot} - S_5)(1 - S_8/(k_9 + S_8)) + S_{5,0} - d_P S_5$
- S6, Nf-κβ: $\frac{dS_6}{dt} = p_5 S_4 (S_{6,tot} - S_6)(1 - S_8/(k_{11} + S_8)) - d_P S_6$
- S7, Smad7 RNA: $\frac{dS_7}{dt} = \frac{g_6 S_5}{(k_6 + S_5)} + \frac{g_7 S_6}{(k_7 + S_6)} + S_{7,0} - d_R S_7$
- S8, Smad7: $\frac{dS_8}{dt} = \frac{h_8 S_7}{(k_8 + S_7)} - d_P S_8$
- S9, MT1-MMP RNA: $\frac{dS_9}{dt} = \frac{g_{12} S_5}{(k_{12} + S_5)} - d_R S_9$
- S10, MMP1 RNA: $\frac{dS_{10}}{dt} = \frac{g_{13} S_6}{(k_{13} + S_6)} - d_R S_{10}$
- S11, MT1-MMP: $\frac{dS_{11}}{dt} = \frac{h_{14} S_9}{(k_{14} + S_9)} - d_P S_{11}$
- S12, MMP1: $\frac{dS_{12}}{dt} = \frac{h_{15} S_{10}}{(k_{15} + S_{10})} - d_P S_{12}$

Table 1. Parameter Definitions

p_1	activation rate of receptor complex TGF-βR when bound and activated by TGF-β1
p_2	activation rate of TAK1 by TNFα and TGF-βR; Includes the binding of TNFa to its receptor complex, which then phosphorylates TAK1; Also includes TGF-B-induced activation of TAK1, which occurs independent of TGF-βR's kinase function [2]
p_3	phosphorylation rate of TGF-βR by Smad2/3
p_5	release rate of active Nf-κβ transcription factors (p50 and p65); TAK1 phosphorylates kinase complex Ikkα Ikkβ Ikkγ, which ubiquitinates a complex containing IkBα and other Nf-κβ transcription factors, which is then degraded, releasing the active Nf-κβ transcription factors
k_6	transcription factor Smad2/3, complexed with Smad4 and interacting with transcription factor Sp-1, binds to Smad7 promoter [3]
k_7	transcription factor Nf-κβ (p50 and p65) binds to Smad7 promoter [3]
k_8	ribosome binds to Smad7 RNA
k_9	Smad7 inhibits phosphorylation of Smad2/3
k_{10}	inhibitor Smad7 inhibits phosphorylation of TAK1
k_{11}	inhibitor Smad7 inhibits Nf-κβ activity
k_{12}	transcription factor Smad2/3, interacting with transcription factor Sp-1, binds to Sp-1 site in MT1-MMP promoter
k_{13}	transcription factor Nf-κβ binds to partial site on MMP1 promoter
k_{14}	ribosome binds to MT1-MMP RNA
k_{15}	ribosome binding to MMP1 RNA
g_6	transcription rate of Smad7 RNA
g_7	transcription rate of Smad7 RNA
g_{11}	transcription rate of MT1-MMP RNA
g_{12}	transcription rate of MMP1 RNA
h_8	translation rate of Smad7
h_{13}	translation rate of MT1-MMP
h_{14}	translation rate of MMP1
d_P	combined protein degradation, dilution, and spontaneous dephosphorylation/deactivation rate
d_R	RNA degradation and dilution rate
$S_{4,0}$	basal expression of TAK1
$S_{5,0}$	basal expression of Smad2/3
$S_{3,tot}$	total steady state amount of TGF-βR in cell
$S_{4,tot}$	total steady state amount of TAK1 in cell
$S_{5,tot}$	total steady state amount of Smad2/3 in cell
$S_{6,tot}$	total steady state amount of Nf-κβ

Note that all "k" parameters represent binding reactions, and are the ratio of the unbinding rate constant to the binding rate constant.

Parameter Fitting

The model was fit to experimental data using gradient descent to determine the parameters for the closest possible fit to the experimental data, given in Table 2. All experimental cytokine and MMP values are expressed relative to the baseline value, which are always expressed as 1.

Table 2. Fit Parameters

Param.	Value	Param.	Value	Param.	Value
p_1	1.00000E+02	k_{12}	4.33120E-01	h_{14}	9.72298E-04
p_2	1.00000E+02	k_{13}	1.11680E+00	d_P	2.20000E-04
p_3	1.00000E+02	k_{14}	1.00093E+00	d_R	1.00000E-03
p_5	1.00000E+02	k_{15}	9.88607E-01	$S_{4,0}$	3.35000E-08
k_6	1.00000E+00	g_6	1.80089E-04	$S_{5,0}$	5.00000E-06
k_7	1.45158E-01	g_7	5.71086E-02	$S_{3,tot}$	1.00000E+00
k_8	1.13724E+00	g_{11}	7.12047E-03	$S_{4,tot}$	1.00000E+00
k_9	4.95965E-07	g_{12}	1.41971E-02	$S_{5,tot}$	1.00000E+00
k_{10}	6.75611E-01	h_8	8.70894E-04	$S_{6,tot}$	1.00000E+00
k_{11}	1.46654E-03	h_{13}	9.72466E-04		

Results

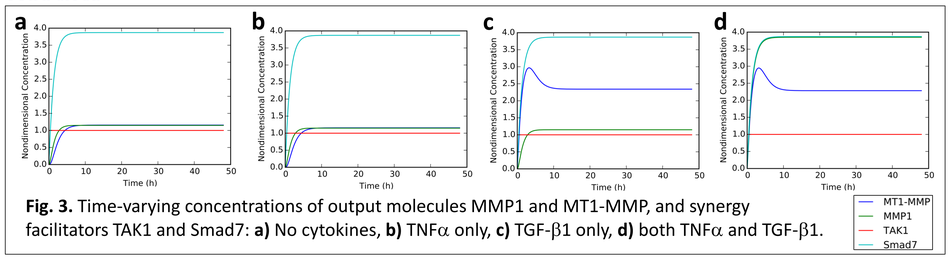


Fig. 3. Time-varying concentrations of output molecules MMP1 and MT1-MMP, and synergy facilitators TAK1 and Smad7: **a)** No cytokines, **b)** TNFα only, **c)** TGF-β1 only, **d)** both TNFα and TGF-β1.

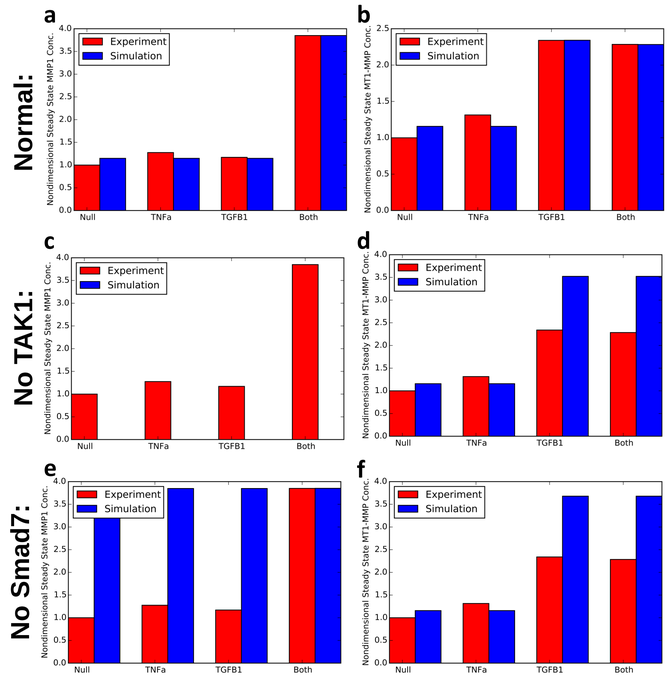


Fig. 4. Comparison between original experimental data and model results: **a)** steady state MMP1 concentration, **b)** steady state MT1-MMP concentration, **c)** steady state MMP1 concentration with no TAK1 signaling, **d)** steady state MT1-MMP concentration with no TAK1 signaling, **e)** steady state MMP1 concentration with no Smad7 signaling, **f)** steady state MT1-MMP concentration with no Smad7 signaling.

a MMP1

Parameter	-10%	No Change	+10%
p1	-1.49E-10	0	2.35E-10
p2	6.54E-09	0	5.45E-09
p3	-1.61E-09	0	-1.30E-09
p5	0.00043723	0	0.00035773
k6	2.39E-09	0	2.22E-09
k7	1.12E-06	0	1.12E-06
k8	8.79E-06	0	8.75E-06
k9	-1.68E-09	0	-1.27E-09
k10	5.46E-09	0	4.67E-09
K11	0.00043706	0	0.0003576
k12	-1.60E-09	0	1.07E-10
k13	-0.0681441	0	-0.0681441
k14	-2.42E-10	0	1.41E-10
k15	-0.1288089	0	-0.1288089
g6	-2.39E-09	0	-2.17E-09
g7	-9.72E-06	0	-7.99E-06
g11	-1.39E-10	0	1.53E-10
g12	0.14312098	0	0.11709898
h8	-0.0003934	0	-0.0003934
h13	-2.36E-10	0	1.89E-10
h14	1.111111111	0	0.90909091
dP	-1.0000001	0	-1.0000002
dR	-0.128801	0	-0.1287993
S4_0	-2.28E-10	0	1.65E-10
S5_0	-8.36E-10	0	-3.24E-10
S3_tot	4.99E-09	0	3.95E-09
S4_tot	0.00043723	0	0.00035773
S5_tot	-1.66E-09	0	-1.51E-09
S6_tot	0.07571432	0	0.06194829

b MT1-MMP

Parameter	-10%	No Change	+10%
p1	6.71E-07	0	5.45E-07
p2	-1.32E-08	0	-8.09E-09
p3	2.94E-01	0	2.55E-01
p5	-5.01E-06	0	-4.08E-06
k6	1.59E-06	0	1.30E-06
k7	7.76E-04	0	7.75E-04
k8	6.10E-03	0	6.08E-03
k9	2.94E-01	0	2.55E-01
k10	-2.79E-08	0	-1.34E-08
K11	-4.95E-06	0	-4.06E-06
k12	-4.11E-01	0	-4.11E-01
k13	1.05E-08	0	-4.93E-09
k14	-4.84E-01	0	-4.84E-01
k15	1.48E-08	0	-6.92E-09
g6	-1.55E-06	0	-1.58E-06
g7	-6.75E-03	0	-5.54E-03
g11	5.37E-01	0	4.40E-01
g12	1.16E-09	0	7.89E-09
h8	-0.2808602	0	-0.2655243
h13	1.11E+00	0	9.09E-01
h14	1.41E-08	0	-8.87E-09
dP	-1.11185	0	-1.1183582
dR	-0.47805	0	-0.4768518
S4_0	1.80E-08	0	-2.68E-09
S5_0	1.19E-01	0	1.12E-01
S3_tot	2.94E-01	0	2.55E-01
S4_tot	-4.98E-06	0	-4.08E-06
S5_tot	3.19E-01	0	2.76E-01
S6_tot	-0.0008615	0	-0.0007052

c MMP1 Synergy

Parameter	-10%	No Change	+10%
p1	0.65249821	0.65249823	0.65249824
p2	0.65249823	0.65249823	0.65249823
p3	0.64602223	0.65249823	0.65826266
p5	0.65144821	0.65249823	0.65338005
k6	0.65249989	0.65249823	0.65249685
k7	0.65344711	0.65249823	0.65157499
k8	0.65329478	0.65249823	0.6517322
k9	0.64602223	0.65249823	0.65826265
k10	0.65249823	0.65249823	0.65249823
K11	0.65144864	0.65249823	0.65337968
k12	0.64649882	0.65249823	0.65776405
k13	0.65249823	0.65249823	0.65249823
k14	0.64721378	0.65249823	0.65709662
k15	0.65249823	0.65249823	0.65249823
g6	0.6524966	0.65249823	0.65249985
g7	0.65165084	0.65249823	0.65321968
g11	0.65757087	0.65249823	0.64772645
g12	0.65249823	0.65249823	0.65249823
h8	0.65982995	0.65249822	0.64569752
h13	0.65249823	0.65249823	0.65249823
h14	0.65249822	0.65249823	0.65249823
dP	0.6407805	0.65249823	0.6631835
dR	0.64794893	0.65249823	0.65627534
S4_0	0.65144788	0.65249823	0.65338032
S5_0	0.66545573	0.65249823	0.64107698
S3_tot	0.64602222	0.65249823	0.65826265
S4_tot	0.65249855	0.65249823	0.65249796
S5_tot	0.645562	0.65249823	0.65867867
S6_tot	0.65147393	0.65249823	0.65335975

Most sensitive: d_P,h_{14},g_{12},k_{15},d_r Most sensitive: d_P,h_{13},k_{14},d_R,k_{12} Most influential: $S_{5,0}$,d_P,h_8,$S5_{tot}$,k_9

Fig. 5. a-b) Sensitivity studies giving elasticities for MMP1 and MT1-MMP output values when the parameter values are varied by 10% in both directions, respectively. The elasticity of the MMP output value is calculated according to e = %Δ[MMP]/%ΔX where [MMP] is the final simulated MMP concentration and X is the parameter value. **c)** MMP1 synergy study to determine which parameters most control the synergistic expression of MMP by TGF-β and TNFα. The synergy is calculated according to $\phi = \log_{10}([\text{MMP1 for TGF-}\beta\text{ and TNF}\alpha]/([\text{MMP1 for TGF-}\beta\text{ only}] + [\text{MMP1 for TNF}\alpha\text{ only}])$.

Conclusions

- Our model recaptures the fundamental system behaviors, namely, the synergistic interactions between the TGF-β and TNFα signaling pathways underlying MMP1 expression.
- Our model supports the idea that TAK1 and Smad7 form the primary linkages between these two signaling pathways allowing for the observed synergy to occur.
- In the absence of either TAK1 or Smad7, the synergistic behavior is lost.
- MMP expression and observed MMP1 synergy is heavily driven by a few important reactions, including protein degradation, translation, and a small number of signaling interactions.

Acknowledgements

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References

[1] 1.Li, R. et al. Macrophage-Secreted TNFα and TGFβ1 Influence Migration Speed and Persistence of Cancer Cells in 3D Tissue Culture via Independent Pathways. Cancer Research 77, 279–290 (2017).
[2] Kim, S. I. et al. Transforming Growth Factor-β (TGF-β1) Activates TAK1 via TAB1-mediated Autophosphorylation, Independent of TGF-β Receptor Kinase Activity in Mesangial Cells. Journal of Biological Chemistry 284, 22285–22296 (2009).
[3] Freudlsperger, C. et al. TGF-β and NF-κB signal pathway cross-talk is mediated through TAK1 and SMAD7 in a subset of head and neck cancers. Oncogene 32, 1549–1559 (2013).